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Progress Report

RESISTANCE OF PINES TO BARK BEETLES

Studies on Toxicity of Resins

1958

By R.H. Smith

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PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION
FOREST SERVICE, U.S. DEPARTMENT OF AGRICULTURE

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PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION
Division of Forest Insect Research

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SUMMARY

This report covers a continuation of research on the project, "The susceptibility or resistance of pines to bark beetles" during the 1958 field season. The work, as in 1956[1/] and 1957[2/], bore heavily on the question of the toxicity of pine resins to bark beetles. In general this work was a refinement of the earlier studies. However there was a distinct change in the procedure used for fumigant toxicity tests because a suitable device had to be found which would completely stopper the fumigation chamber and yet not absorb the resin vapors. Such a container was necessary to obtain accurate data on the concentration of the vapors in which beetles were confined.

The results of several facilitating tests were quite helpful in determining the course of subsequent fumigation experiments. It was found there was little difference in the mortality rate of beetles which were observed repeatedly and those which were observed only once. Therefore data from repeated observations on the same lots of beetles were considered valid. Cork-, neoprene-, and polyethylene-stoppered test tubes, as well as weighing bottles with ground glass unions, were unable to suitably contain resinous vapors. However, a 4-oz. jar with a screw-cap and a teflon gasket was able to contain resinous vapors completely.

1/ Smith, R.H. 1959. Progress report, resistance of pines to bark beetles, studies on toxicity of resins, 1956. Calif. Forest & Range Expt. Sta. 43 pp. illus. (processed)

2/ ________ 1959. Progress report, resistance of pines to bark beetles, studies on toxicity of resins and response of beetles, 1957. Pacific Southwest Forest & Range Expt. Sta. 43 pp. illus. (processed)

The 4-oz. jar was selected as the basic testing device for fumigation tests. The suitability of the materials to be used in the resinous vapor atmosphere was based on their nonabsorptiveness of the vapors. Suitable materials were teflon, lumite, and nylon; unsuitable ones were cork, neoprene, and polyethylene.

Determinations were made of some of the physical properties of resins which might influence the conduct of the work as well as the fumigant toxicity of resin vapors. The volatile fraction of ponderosa and Jeffrey pine and the Jeffrey x ponderosa hybrid was found to be approximately 25, 15, and 19 percent respectively at 70°F. The vapor saturation in a 150 cc. volume was approximately 2.0, 20.0, and 6.0 milligrams respectively and the time to reach this saturation was about 12 hours. As the season's work was reviewed, though, it was apparent that there was considerable variation in these figures despite efforts to maintain uniformity in the procedure.

A series of tests was made to determine the effect on Dendroctonus brevicomis Lec. of saturated and subsaturated concentrations of resinous vapors of ponderosa, Jeffrey, and hybrid resin. Generally, these tests showed that ponderosa had no fumigant toxicity effect even at saturation with a 7-day exposure. However in a few instances toxicity was apparent where, from some unexplained reason, the vapor saturation of ponderosa resin was much higher than average. With Jeffrey pine resin, a 3-day exposure to a saturated atmosphere caused 100 percent mortality. Jeffrey pine resin vapor concentrations were nontoxic below 5.0 mg. but toxic above 10.0 mg. The hybrid resin was intermediate between the two parents. It was nontoxic at subsaturation concentrations but toxic at saturation with long exposures.

Limited work with Dendroctonus jeffreyi Hopk. indicated that ponderosa resin was toxic to it at vapor saturation, but Jeffrey resin was nontoxic at either saturation or at subsaturation. Likewise results with the hybrid resin indicated some toxicity at saturation but no toxicity at subsaturation.

The results of tests with these two species of beetles point to the conclusion that each beetle species is unaffected by the resin vapor of its recognized host but is adversely affected by the resin vapors of its nonhost tree. Neither beetle appears to be able to tolerate high concentrations of hybrid resin vapor for extended periods of time.

The season's work with ponderosa resin and its derivatives again clearly demonstrated that commercially prepared turpentine was neither physically nor biologically synonomous with the natural liquid fraction of fresh resin.

Contrary to the findings in the 1957 tests, where a cork-stoppered test tube was used as a fumigation chamber, humidity was found to have no effect on either the fumigant toxicity of resin vapors or on the rate of natural mortality of D. brevicomis. The lack of agreement between the 1957 and 1958 tests necessitated a comparison of the two under as

equitable conditions as possible. The results showed a distinct difference. Beetles in a cork-stoppered test tube, as used in the 1957 experiments, showed not only a toxic response to ponderosa resin vapors but also a faster rate of natural mortality than unconfined beetles. Beetles in a 4-oz. jar, as used in 1958, had the same rate of natural mortality as unconfined beetles and only rarely showed a toxic response to ponderosa resin vapors. The results obtained in 1957 are considered questionable because of the uncertain action of cork and the lack of accurate data on the weight of vapor in the chamber. These deficiencies were eliminated in the 1958 tests through the use of a teflon gasket and through the determination of the actual weight of vapor in the fumigation chamber.

Semi-confined vapors of the three resins had no effect on the feeding behavior of *D. brevicomis*.

Contact toxicity experiments were more extensive than in 1957 and larger numbers of beetles were used. The results indicated that a complete coating of ponderosa or Jeffrey resin caused a significant increase in the rate of mortality of both *D. brevicomis* and *D. jeffreyi*. Beetles of both species which were treated with ponderosa resin showed an increased ability or inclination to feed on bark. With a coating of Jeffrey resin, *D. brevicomis* reacted unfavorably, while *D. jeffreyi* reacted favorably with respect to feeding ability or inclination.

Tests of the stomach toxicity of resin were made by allowing *D. brevicomis* to feed on strips of bark impregnated with different resins and resinous materials. Though the data are not extensive, it does appear that the amount of feeding on bark treated with natural resinous materials was far less than on untreated bark; however, the rate of mortality was not altered. Bark treated with turpentine appears to have stimulated feeding but at the same time appears to have caused an increase in the mortality rate of beetles. This is another instance where natural resin materials differed from commercial derivatives.

INTRODUCTION

This report represents a continuation of the research on the question of the susceptibility or resistance of pines to bark beetles. The factor of toxicity of resins again received major attention. The general objective of the work remained unchanged. Essentially it is to determine if and how resin can play a decisive role in the success or failure of a bark beetle attack on a pine tree.

Economically this is an important question. The continued emphasis on the improvement of forest trees through selection and hybridization will in time change the composition of forest stands. The determination of the role of resin in tree resistance to insect attack could aid in a more intelligent selection and breeding of trees economically suited to an area. It may be some time before such information is available. However an interim value, which may be realized from this research, is the entomological evaluation of species and hybrids used or developed by a tree-improvement program.

The ecological implications of such studies could be of even greater fundamental importance. The question of the relationship between bark beetles and resins is of basic value toward a better understanding of management of pine stands in the face of potential or actual bark beetle attack. An intimate association has long been recognized between resin-producing trees and spectacular, widespread outbreaks as well as persistent endemic situations of bark beetles. Resin is, at present, considered a by-product of plant metabolism and plays little or no part in the basic functions of growth and reproduction. Opinions have been expressed that ascribe both a beneficial and deleterious role to such secondary plant substances. Fraenkel[3] believes that the primary role of such secondary plant substances is entomological. Mirov[4] records a diversity of volatile secondary substances in pine resins.

The general course of the work was determined by the two previous seasons' findings. Again the emphasis was on fumigant toxicity of the resin of ponderosa and Jeffrey pine, and a hybrid of these two pines to the western pine beetle and the Jeffrey pine beetle. The basic technique was altered to enable an accurate determination to be made of the concentration of the resin vapors. Some effort was devoted to the question of contact toxicity, though techniques for studying contact toxicity and for evaluating results still presented difficulties. A small exploratory test was made of stomach toxicity of resin and resin derivatives. The work on insect response was insufficient to be reported. Lack of suitable techniques continues to hamper this type of study.

The work was carried out at the Institute of Forest Genetics at Placerville. The facilities and, upon a few occasions, the personnel of the Institute were made available to the work. Mr. Barry Pullen, a graduate student in entomology at the University of California, assisted in the collection of the field data.

GENERAL PROCEDURE

Beetles

Beetles were reared from infested material placed in the insectary at the Institute. The problem of high midday temperature was again encountered in the insectary and partially, though not satisfactorily, solved by placing an evaporative cooler at one end of the roof gable and directing the air downward and over the insectary units.

3/ Fraenkel, G.S. 1959. The raison d'être of secondary plant substances. Science 129 (3361): 1466-70.

4/ Mirov, N.T. 1959. The fragrance of pines. Atlantic Monthly 204(3): 60-62.

TABLE OF CONTENTS

Dendroctonus brevicomis Lec. brood material was obtained from the bark of infested trees located in the vicinity of the Institute of Forest Genetics and the Eldorado National Forest. The bark was removed from the trees when the brood was in the last larval stage and was held in cold storage at 35°F. until needed. Then it was placed in the insectary. Adults were collected twice daily in individual gelatin capsules and held at 35°F. until ready for use in tests. Collected beetles held in refrigeration for more than 48 hours were not used. Just prior to their use, all beetles of a test were equitably distributed by age and size among all the replicates of the test.

Dendroctonus jeffreyi Hopk. brood material was obtained from two infested trees about 3 feet d.b.h. One was located on the Eldorado National Forest and the other on the Toiyabe. One tree was felled when the brood was in the late larval and early pupal stage. It was cut into 15-inch to 18-inch lengths. Four slabs were ripped from each of these lengths with a chain saw. Each slab consisted of the bark and an arc of sapwood with a maximum thickness of 6 inches. With this procedure it was not necessary to handle the great bulk of the wood of the tree. Most slabs could be easily handled by one man. The system worked well and is recommended for work with bark beetles in large trees. Leaving the great bulk of the wood at the tree site not only alleviated the handling of the brood material but also enabled a greater amount of the material to be placed in a unit of the insectary. This is an important point with this particular beetle because it emerges slowly over an extended period of several weeks. Thus it is difficult to obtain sufficient numbers to conduct the designed tests, unless a large amount of brood material is placed in the insectary at one time. The second tree was handled quite differently. By a fortuitous coincidence the tree was cut just as the brood was beginning to emerge and a great many beetles were in the process of boring out through the thick bark. The bark at this time was too loose to permit the bolts to be slabbed like the other tree. Only the bark, containing the beetles in the process of boring out, was taken. In a sense the bark was handled like the *D. brevicomis* brood material. It is doubted if many trees can be cut at the right time for the last procedure to be of much value. Nevertheless it certainly should be used where possible because of the tremendous saving in time, space, and effort.

Resin

Resins were extracted from the trees as in previous seasons by making a circular punch wound a short way into the sapwood of a tree. A tubular plastic tap with a collecting vial on the lower surface was fitted into the wound. The same four trees were used in all tests: one ponderosa pine (*Pinus ponderosa* Laws.), one hybrid pine (*Pinus jeffreyi* x *ponderosa*), and two Jeffrey pines (*Pinus jeffreyi* Grev. & Balf.) of the same parentage. In all cases except where noted fresh uncrystallized resin was used.

Recording and analysis of data

Usually the only observation made was of the condition of the beetle. A beetle was considered dead when it did not move when agitated. This

information was kept separately for each beetle whose sex and size was determined post mortem if these data were considered desirable. The results were first visually inspected. If it was considered necessary, an analysis of variance with the t-test was made to determine if the observed differences were significant.

FUMIGANT TOXICITY
--FACILITATING EXPERIMENTS--

Data in the following 5 sections were gathered primarily to determine the procedures and materials to be used in the fumigation experiments. Data presented in the first 4 sections were gathered prior to undertaking any fumigation experiments. The data in the fifth section, "physical properties of resin," were gathered in the course of subsequent fumigation tests.

Repeated vs. single observations of beetles

The 1956 and 1957 procedure included repeated, usually daily, observations of the same replicates of beetles. Doubt had been expressed as to the validity of data obtained from such observations. Likewise, at the present, there are no statistical methods which permit a complete analysis of such data, although they can be analyzed within but not between time intervals.

Therefore a test was established to compare the mortality rate of *D. brevicomis* under conditions of repeated and single observations. The standard cork-stoppered 30 cc. test tube was used with 12 beetles per tube. As in the 1957 tests, the 12 beetles were held in 3 bundles of 4 cells each (figure 3a). Set A, consisting of 64 tubes, was divided into 8 groups of 8 replicates each and observed for natural mortality. Two groups were observed daily while each of the remaining 6 groups was observed only once during the 6-day period. Set B, consisting of 64 tubes, was assembled a few days later and divided into 8 groups of 8 replicates each. All replicates in set B were held without observation for 7 days after which one group was observed daily and each of the remaining 7 groups was observed only once during the ensuing 7-day period. There were two lots of beetles for the 13th day observations. Thus the two sets covered a period of 13 days.

A summary of the results is given in table 1, while table 2 is a realignment of the data of set A according to sex. Since set B was terminated on the 13th day, table 3 is a breakdown by sex of beetles which were living on that date for the 8 groups of beetles. Figure 1 is a size distribution by sex of the beetles in each of the two sets. Figure 2 is the mortality rate by size of beetle.

Discussion.--The frequency of observation had no significant effect and very little apparent effect on the natural mortality rate of *D. brevicomis*. Therefore data from repeated observations of the same

lots of beetles probably can be considered valid. Females and larger beetles had a slower mortality rate than males and smaller beetles respectively. On an average females were larger than males. Thus, since beetles were equitably distributed by size among all replicates of a test, variations which might be introduced by sex were probably offset by size.

Table 1.--Cumulative natural mortality of *D. brevicomis* in 30 cc. test tube with different frequencies of observations

Frequency of observation	Total beetles used	Days after start of test: 1	2	3	4	5	6	7	8	9	10	11	12	13
	Number	---Number---												
Daily	64	1	3	6	10	16	21	24	31	34	42			
Daily	64	0	3	6	9	13	15	22	35	39	44			
Once	384*	-	2	4	6	14	20	29	-	-	-			
Daily	64	-	-	-	-	-	-	25	30	38	42	47	53	57
Once	384*	-	-	-	-	-	-	-	20	33	33	44	41	57
Once	64	-	-	-	-	-	-	-	-	-	-	-	-	53

Comparison	t-value
Daily vs. once on the 4th day =	0.755
8th day =	1.333
12th day =	1.781
t at 90% =	1.753

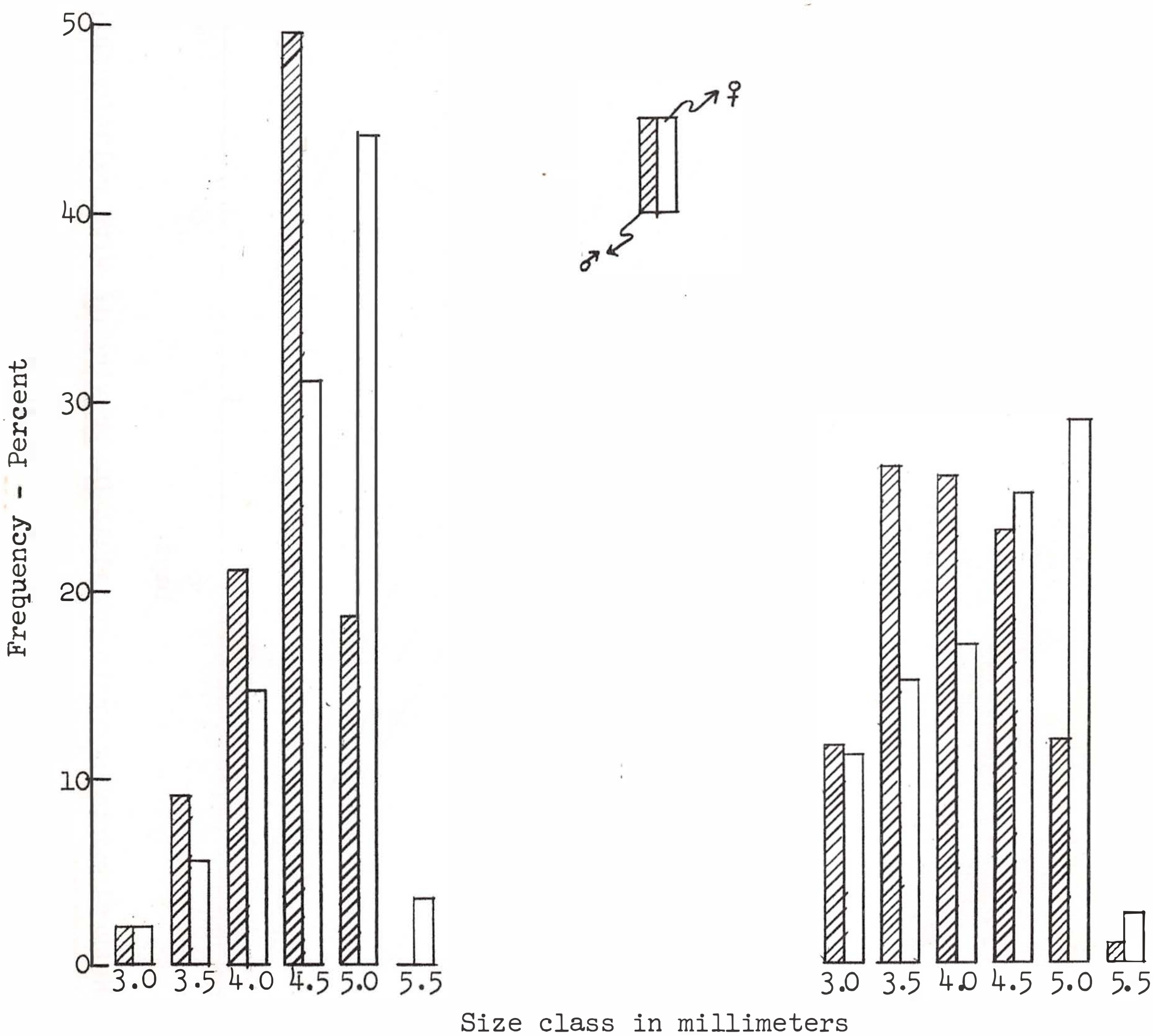
* Of the 384 beetles, only one set of 64 was chosen at random and observed each day.

Table 2.--Cumulative natural mortality of male and female *D. brevicomis* in the 30 cc. test tubes of set A

Sex	Total beetles used	Days after start of test								
		2	3	4	5	6	7	8	9	10
	Number	---Percent---								
Female	285	1.0	3.5	7.4	12.6	20.0	28.4	38.5	45.6	56.1
Male	212	2.9	4.8	7.1	14.3	25.7	42.4	57.1	64.7	79.0

Table 3.--Percent of males and females alive after the 13th day in set B

Sex	Day observations were begun							
	7th	8th	9th	10th	11th	12th	13th	13th
	---Percent---							
Female	23.0	35.7	27.3	29.4	16.7	36.8	19.4	21.6
Male	5.3	9.1	6.5	7.4	2.9	15.4	3.1	7.4



Set A		Set B	
Number of beetles	Average length	Number of beetles	Average length
285	4.6 mm	259	4.3 mm
217	4.4 mm	251	4.0 mm

Figure 1.--Distribution of sizes by sex of adult *D. brevicomis*.

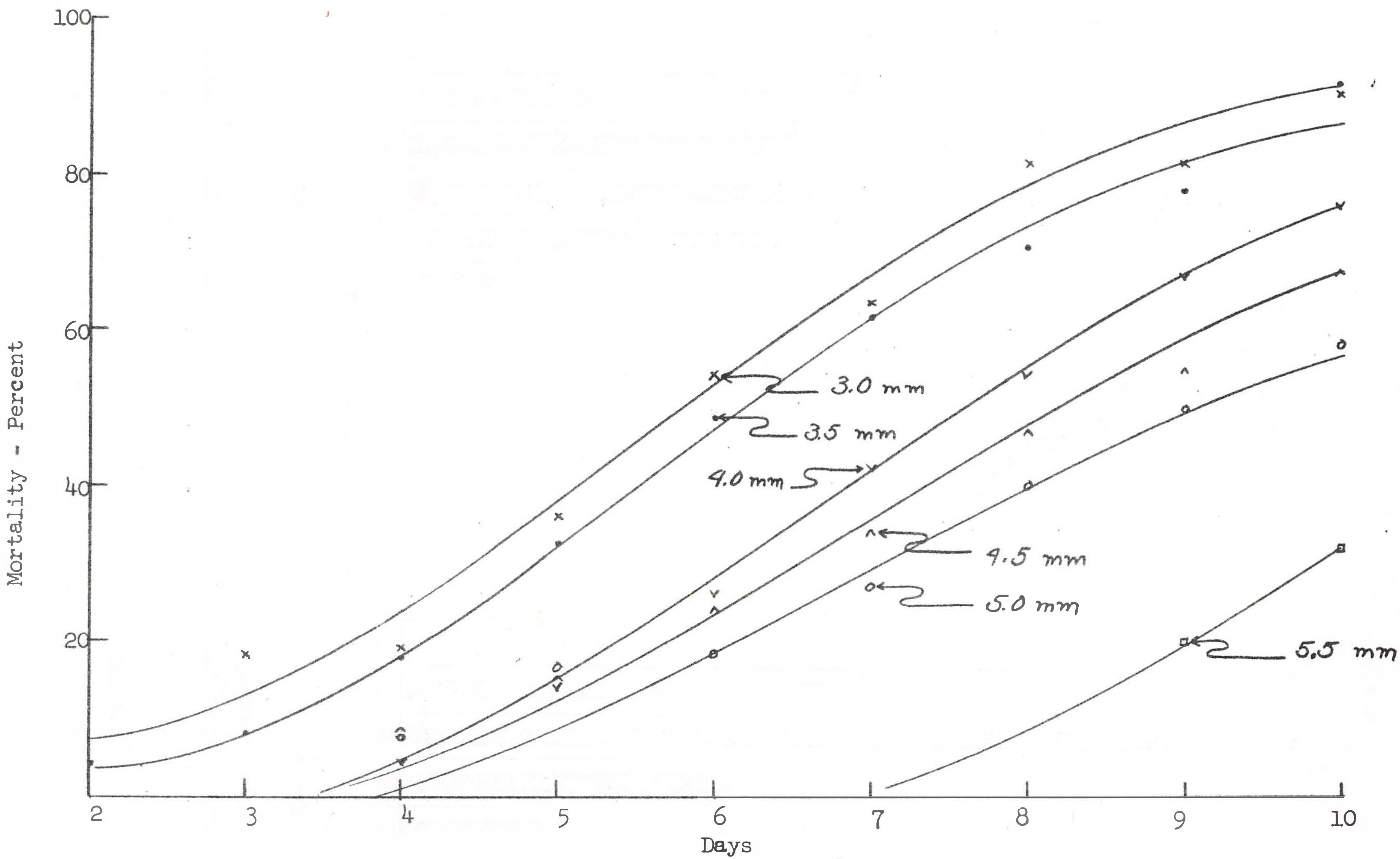


Figure 2.--Natural mortality rate of adult D. brevicomis with relationship to length of beetles.

Fumigation chambers

From the standpoint of technique, one of the most difficult problems has been the selection of a device which would confine resin vapors without absorbing them. Before the 1957 field season began, efforts were made to solve this problem. Three types of devices were tried as follows:

1. Weighing jar with ground glass unions.
2. 30 cc. test tube stoppered with various materials.
3. One-ounce screw-cap jar with teflon gasket.

A small amount of α-pinene was placed in the weighing jars. The ground glass surfaces were united without sealing compound, with sealing compound, and with a thin strip of polyethylene. A series of weighings indicated that none of these were effective in completely containing the α-pinene. Therefore chambers sealed with ground glass unions were considered unsatisfactory.

The effectiveness of containment in the test tube and the one-ounce jar was measured by the change in level of α-pinene in a $\frac{1}{4}$-dram (4 mm. i.d.) vial which was placed in the confined atmosphere. The $\frac{1}{4}$-dram vials were marked with reference lines about 1 mm. apart. A continued lowering of the level of the α-pinene in the small vial after a 2-day period, which was sufficient time for the confined atmosphere to become saturated, was accepted as evidence that the volatile material was either escaping or being absorbed by the stoppering material.

The test tubes were stoppered with both neoprene and cork. Each was used alone and with a wrapping of polyethylene or teflon. None of the six combinations were effective in confining the α-pinene in the test tube. Therefore the use of test tubes was discontinued.

The 1-oz. jar with a screw cap and a teflon gasket proved to be highly effective in containing resin vapors. Several months after the jars were assembled there was still no loss of vapor as indicated by the level of the liquid. Occasionally there was a drop in the level but in every case this was caused by recondensation of the gaseous α-pinene within the jar. In jars where this recondensation did not take place the liquid level remained constant. Recondensation seemed to be caused by fluctuating temperatures over a several week period; therefore it is not a factor to consider in the fumigant tests which are conducted for short periods of time at a constant temperature.

As a result of this work, the screw-cap jar with a teflon gasket was selected as the basic test unit for the 1958 fumigant tests. A 4-oz. jar was chosen because its size seemed most suitable for the other materials which were used in the tests.

Absorption of resin vapors

The question of complete containment of resin vapors was answered by the previous test with different types of fumigation chambers. From

this experiment it also seemed evident that teflon was not absorbing the vapors. However, it was thought advisable to determine this with more certainty by conducting a test in which teflon and other materials used in fumigation chambers were allowed to remain in a saturated resinous atmosphere for several days.

The 4-oz. screw-cap jar was used with a teflon disk as a gasket. An approximate volume of 1.0 ml. of ponderosa resin was apportioned with a pipette into a 2 ml. resin vial. This volume of resin was more than adequate to produce a saturated atmosphere of vapor in the 4-oz. jar. The materials to be tested were placed in the 4-oz. jar along with the small vial of resin. Three jars were disassembled on each of the 1st, 2nd, and 7th days after the test was started. The materials were reweighed and the difference between the initial weight and the re-weight, corrected for any change in weight of untreated checks, was considered "pickup" of vapor by the material.

Later a second test using Jeffrey pine resin was made with those materials which did not absorb ponderosa resin.

The results which are summarized in table 4 show the high absorptiveness of neoprene, the uncertainty of cork and the rather low absorptiveness of polyethylene. The nonabsorptiveness of teflon, nylon, and lumite are quite evident. On the basis of this test it was considered safe to use teflon, lumite, and nylon with either ponderosa, Jeffrey, or Jeffrey x ponderosa pine resin.

4-oz. jar procedure

The change to the 4-oz. jar fumigation chamber necessitated a revision in the procedure followed in setting up a test. The steps taken in assembling a test with the 4-oz. jar are as follows (figure 3):

a. A replicate of 12 beetles was taken from refrigeration.

b. Four of these beetles were transferred from their individual gelatin capsules into the 4 cells of a bundle (figure 3a). Each opening of the 4 cells was "plugged" with a piece of lumite mesh (figure 3b).

c. The other 8 beetles were placed in the cells of 2 other bundles, thus making 3 bundles of 4 beetles each.

d. These 3 bundles were carefully placed in a stack (figure 3c) with the "plugged" bundle on top. In this way beetles were confined to the cells by the bundle immediately above them. The beetles in the top bundle were confined by the "plugs."

e. The stack was securely bound with a single piece of nylon thread. After this, the stack was easily handled as a single unit. Each stack was considered a replicate.

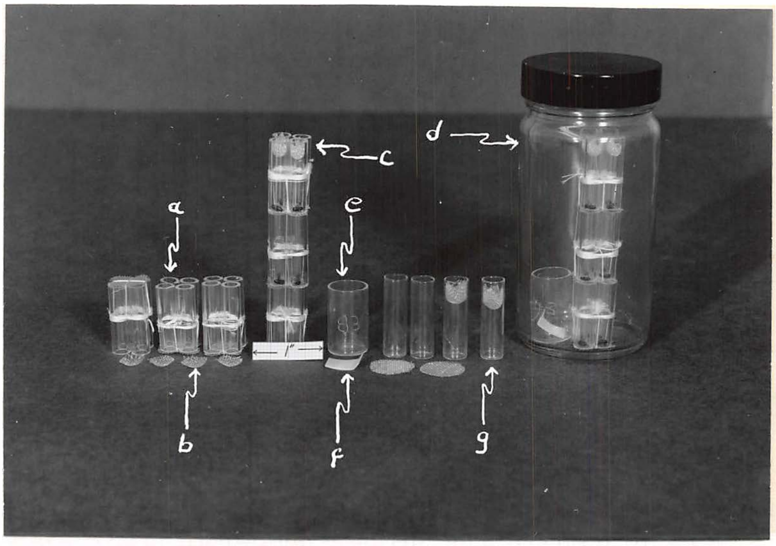


Figure 3.--Four-ounce jar and materials for fumigation test.

a. Bundle of 4 cells.

b. Lumite "plug" for top bundle of cells.

c. "Stack" of 3 bundles bound with nylon.

d. 4-ounce jar with "stack" and resin vial within.

e. Resin vial.

f. Teflon square.

g. "Plugged" 1/4-dram vial.

Table 4.--Absorption of resinous vapors by various materials in a saturated atmosphere after different periods of confinement

Resin	Material	Approximate weight of material	Days of confinement 1	2	7
		Milligrams	---Milligrams---		
Ponderosa	cork	250.0	2.2	0.2	0.6
	neoprene	2,200.0	11.8	22.1	68.1
	polyethylene	50.0	0.6	1.3	1.9
	teflon	150.0	0.0	0.0	0.0
	lumite	150.0	0.0	0.0	0.0
	glass	180.0	0.0	0.0	0.0
	nylon	170.0	- -	0.0	- -
Jeffrey	lumite	350.0	0.0	0.0	0.0
	teflon	120.0	0.0	0.0	0.2
	nylon	160.0	- -	0.0	- -

f. After the necessary stacks had been assembled, they were placed in the proper number of 4-oz. jars to satisfy the requirements of the particular test (figure 3d). In some tests only two of the cells of the top bundle were used in order to produce a replicate of 10 beetles instead of 12. It was found that 12 or 24 beetles, that is one or two stacks, could be confined in one jar for 10 days without changing the rate of natural mortality. Since all tests were less than 10 days, two stacks were sometimes placed in a single 4-oz. jar. In this case each stack was considered a replicate.

g. As soon as the stacks were in the 4-oz jars, the resin was apportioned as follows.

(1) Volumes greater than .01 ml. were apportioned into standard resin vials (figure 3e) with a pipette.

(2) Smaller volumes were apportioned by turning a micrometer shaft against the plunger of a hypodermic syringe. The resin droplet which formed at the end of the needle was "picked up" with a $\frac{1}{4}$-inch square of teflon (figure 3f), which was handled with forceps, and quickly dropped into a standard resin vial.

h. As soon as the resin was in the resin vial it was placed in a 4-oz. jar as quickly as possible, usually within 2 to 5 seconds.

i. The jar, now containing one or two "stacks" of beetles and the resin, was very tightly capped and placed under the conditions of the test.

j. Usually samples of the various volumes and kinds of resin used in a test were placed in jars without beetles. The vials containing these samples of resin were quickly stoppered and weighed before being placed in the 4-oz. jar. Subsequent weighings were made to determine the weight of volatile materials in the 4-oz. jars. These data were applied to the jars containing beetles as an expression of dosage.

k. The jars were disassembled at specified times and the stacks were retained for periodic observations of beetle mortality.

l. At the conclusion of the test the stacks were broken apart and each beetle recorded for size and sex.

m. All tests were held in the dark at $70^{\circ}F. \pm 2^{\circ}$.

Physical properties of resins

Before fumigant tests could be properly conducted it was necessary to determine the weight of vapor necessary to saturate the atmosphere in the 4-oz. jar, the percent volatility of the resin, and the rate of vaporization. Tests which were made to obtain these data were subsequently supplemented by data gathered during the course of the various experiments. When all these data were analyzed, variations in all three properties were evident. Such variations could have been caused by the handling of the resin or by seasonal changes in the properties of resin. The data were insufficient to determine which was the cause. Despite all efforts to standardize the handling of the samples, there could have been sufficient discrepancies in the procedure to cause part or all of the observed fluctuations. However there is some evidence that the properties of resin do fluctuate from time to time.

The determination of all three properties was made through measurement of the loss of weight of resin samples with an analytical balance. The assumption was made that all change in weight was caused by vaporization of part of the resin. Actually this assumption is open to dispute because there is a possibility that some of the solid fractions of resin oxidize and therefore may increase in weight. The short period of the test might minimize this possibility when working with gross resin; however oxidation was probably a major factor in the test where fresh resin was compared with

turpentine. The scope of the work hardly permits a differentiation between weight loss from vaporization and weight increase from oxidation or other chemical actions.

The data from all measurements pertaining to weight loss required to reach saturation are summarized in table 5. Figure 4 represents the weight loss curves for the three resins. The heavy broken lines are generalized curves while each thin solid line represents serial measurements of different sets of samples of the same extraction of resin. Plotted points not associated with lines are nonserial measurements of extractions of resin used for various phases of the testing program. Within a set of measurements there was little or no variation in weight loss.

Table 5.--Weight loss of ponderosa, Jeffrey, and hybrid pine resin after 24 hours or more at 70°F.±2°F. in 150 cc. volume

Date	Ponderosa		Jeffrey		Hybrid	
	Measurements	Average	Measurements	Average	Measurements	Average
	Number	Milligrams	Number	Milligrams	Number	Milligrams
June 20	9	2.0	9	20.9	8	6.2
Jul. 23	16	2.0	15	23.1		
23	14	1.9				
28			3	21.5		
31			3	17.7		
Aug. 2	2	2.4	2	19.6		
6	2	2.5	2	20.0		
11	7	1.8				
12	5	3.4				
13	5	2.0				
14					3	5.2
15					3	6.6
18	2	2.8				
19	4	2.3				
20					6	5.8
28	3	1.7				
Sept. 1	6	1.9	3	15.2		
10			10	15.7		
16	3	1.8				
Average		2.1		19.9		6.0
Range		1.6 to 3.5		14.4 to 25.3		4.6 to 6.8

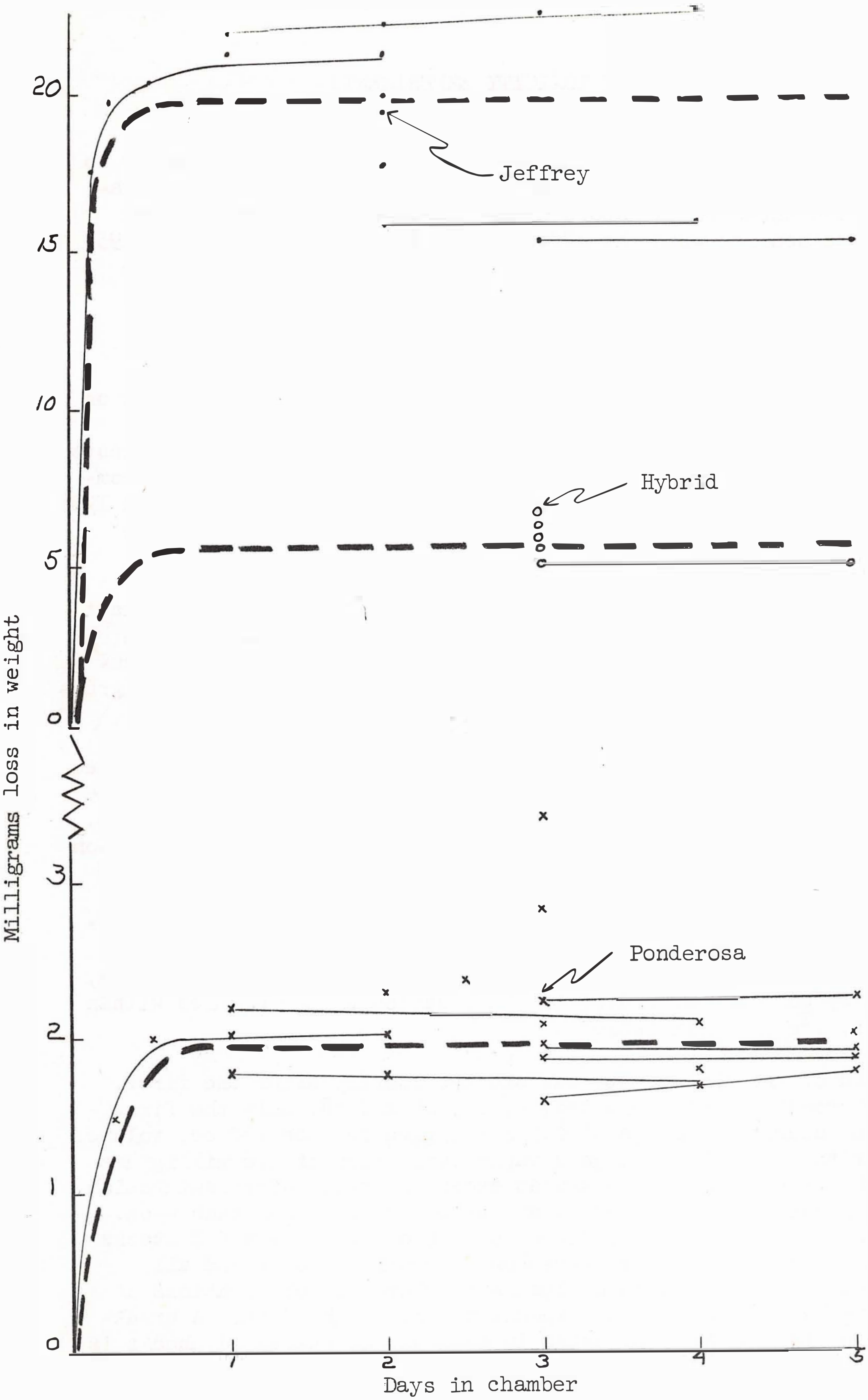


Figure 4.--Weight loss of three pine resins in 150 cc. fumigation chamber at 70° F. $\pm$ 2°.

FUMIGANT TOXICITY EXPERIMENTS

The experiments reported in this section were conducted primarily to associate the mortality of the two beetles with definite concentrations of the three resin vapors under standard conditions. Three other tests dealing with the effect of humidity, comparisons of 1957 and 1958 procedures, and the effect of fumigation on feeding, are included at the end of the section.

Dendroctonus brevicomis with ponderosa pine resin

Three experiments were conducted to determine the fumigant effect on D. brevicomis of various dosages of ponderosa pine resin vapors. Other data pertaining to this beetle and resin were obtained in tests having other primary objectives such as the effect of moisture, comparisons of resin and turpentine, and comparisons of the 1957 and 1958 techniques. These tests are reported later under the appropriate headings.

The standard procedure was used for all three tests. However, exact determination of the weight of vapor in the 4-oz. jar was made in only the third test. In the first two tests this weight was calculated by using a factor 0.15 to convert micrometer units to milligrams of vapor.

In the first test, micrometer units of 3, 6, 12, and 24 were selected in an attempt to get vapor dosage below and at saturation. When the factor of 0.15 was applied to these, vapor dosages of 0.5, 0.9, 1.8, and 3.6 milligrams were obtained. Since 1.8 milligrams is saturation for ponderosa resin in the 150 cc. volume of the 4-oz jar, only 1.8 milligrams is calculated for the 24 micrometer units. There were 12 beetles per replicate and 4 replicates for each dosage. All jars were disassembled after 7 days and the replicates held in a clean atmosphere for observations at 2-day intervals. The mortality data, arranged by dose and according to the location of the beetles within the stack, are given in table 6.

The second of the three tests was started shortly after the first. Of the micrometer units selected, 6, 12, 24 and 48, only the first gave a subsaturation dosage of 0.9 milligrams for the 150 cc. volume. The other three volumes all gave vapor saturation of 1.8 milligrams but in the highest two there was an excess of resin after saturation had been reached. The factor of number of "stacks" for each 4-oz. jar was studied by putting 1 stack in half of the jars and 2 stacks in the other half. All jars were disassembled at 3 days and all replicates were retained in a clean atmosphere for observations at 3-day intervals. Table 7 is a summarization of the data. A breakdown of the individual replicates in each jar of untreated checks is also given in this table to illustrate what might be called the inherent natural variation of the test procedure.

The third test included the general range of micrometer units used in the previous two tests. However, samples of each treatment were

weighed to give the actual rather than the calculated vapor dosage in the 4-oz. jars. Micrometer units of 10, 20, and 40 were used along with a macro volume of 0.2 ml. The latter was apportioned by pipette. Although the resin samples used to obtain the actual weight of vapor were reweighed at 48 hours, previous work indicated that it was safe to assume that conditions at 48 hours represented conditions throughout the period of the test. There were 6 replicates of 12 beetles each, with a complete series of replicates for each dosage disassembled at 3, 5, and 7 days. The replicates were retained in a clean atmosphere and were observed at 2-day intervals. Table 8 is a summary of the data.

Table 6.--Cumulative mortality of D. brevicomis, confined in 150 cc. volume, at calculated vapor dosages of ponderosa pine resin and at different heights in the resinous atmosphere

Micrometer units	Calculated weight of vapor 1/	Total beetles used	Days after start of test: 7	9	11	13	15
	Milligrams	Number			---Number---		
3	0.5	48	8	17	20	32	39
6	0.9	48	12	17	32	39	46
12	1.8	48	7	19	28	40	43
24	1.8	48	15	23	33	45	47
0	0.0	48	12	22	26	42	45
Position within stack							
	top	80	16	30	45	69	-
	middle	80	19	33	42	59	-
	bottom	80	19	35	52	69	-
Comparisons			t-value				
0 vs.	3		.548	.427	.605		
	6		.000	.530	.868		
	12		.898	.304	.224		
	24		.503	.109	.717		
24 vs.	3		1.259	.704	1.425		
	6		.632	.965	.164		
	12		2.087	.557	.614		

t at 90% = 1.895

1/ The 1.8 mg. dose is considered saturation. Doses below this were computed by multiplying 0.15 times the number of micrometer units.

Table 7.--Cumulative mortality of D. brevicomis, confined in 150 cc. volume, at calculated vapor dosages of ponderosa pine resin

Replicates per 4-oz. jar	Micrometer units of resin	Calculated weight 1/ of vapor	Total beetles used	Days after start of test: 3	6	9	12	15
Number		Milligrams	Number	----Number---				
1	0	0.0	36	2	5	14	27	32
	6	0.9	36	0	4	16	28	32
	12	1.8	36	4	9	16	29	36
	24	1.8	36	5	9	20	32	36
	48	1.8	36	4	12	24	33	35
2	0	0.0	72	2	15	30	54	69
	6	0.9	72	1	12	33	57	69
	12	1.8	72	6	16	32	58	70
	24	1.8	72	4	18	47	63	71
	48	1.8	72	4	20	49	70	72
2	0	0.0	12	1	2	5	10	11
	0	0.0	12	0	3	6	9	11
	0	0.0	12	1	3	5	10	12
	0	0.0	12	0	3	6	10	12
	0	0.0	12	0	2	2	6	11
	0	0.0	12	0	2	6	9	12

Comparisons — t-values

	1 stack per jar: 3 days	6 days	2 stacks per jar: 3 days	6 days	Composite of all jars: 6 days
0 vs. 6	2.012	.213	.443	1.022	.739
12	.669	2.000	1.422	.306	1.002
24	1.511	2.000	.613	.624	1.252
48	.669	1.876	.793	.929	1.770
t at 90%	2.015		1.796		1.740

1/ 1.8 milligrams is considered saturation. Doses below this were computed by multiplying 0.15 by the number of micrometer units.

Table 8.--Cumulative mortality of D. brevicomis, confined in 150 cc. volume, at different vapor dosages of ponderosa pine resins

Volume of resin 1/	Weight of resin	Weight of vapor	Total beetles used	Days after start of test: 3	5	7	9	11
	---Milligrams---		Number		---Number---			
10 m.u.	4.4	1.2	72	6	11	42	67	70
20 m.u.	9.9	1.7	72	5	12	39	66	68
40 m.u.	18.1	1.8	72	4	20	40	60	68
.2 ml.	224.3	1.9	72	8	18	42	66	70
0	0.0	0.0	72	4	17	43	67	71
10 m.u.	4.4	1.2	72		12	35	61	70
20 m.u.	9.9	1.7	72		13	41	60	68
40 m.u.	18.1	1.8	72		14	33	62	71
.2 ml.	224.3	1.9	72		22	51	66	70
0	0.0	0.0	72		10	34	54	69
10 m.u.	4.4	1.2	72			27	58	68
20 m.u.	9.9	1.7	72			28	63	71
40 m.u.	18.1	1.8	72			33	60	68
.2 ml.	224.3	1.9	72			42	66	71
0	0.0	0.0	72			35	58	68

Comparisons		t-value		
3-day exposure: 0 vs. 1.2 mg.	.512	1.005	.652	.677
1.7	.136	.592	.984	.399
1.8	.000	.402	.874	1.198
1.9	.789	.148	.472	.299
5-day exposure: 0 vs. 1.2		.354	.073	1.016
1.7		1.014	.672	1.002
1.8		.647	.163	1.230
1.9		1.695	1.791	2.242
7-day exposure: 0 vs. 1.2			1.015	.000
1.7			1.123	.622
1.8			.405	.188
1.9			.900	1.258
Check: 3 day vs. 5 day		.956	1.229	1.727
7 day			1.661	1.021
10 micro. units: 3 day vs. 5 day		.289	1.477	1.369
7 day			2.064	2.475
20 micro. units: 3 day vs. 5 day		.289	.244	.707
7 day			1.708	.000
40 micro units : 3 day vs. 5 day		.978	1.619	.439
7 day			2.484	.000
0.2 ml. : 3 day vs. 5 day		.507	1.057	.000
7 day			.000	.000

t at 90% = 1.796

1/ m.u. = micrometer units; ml. = milliliters.

Discussion.--If all three tests are considered, it would appear that there was a general lack of consistently significant mortality by the vapors of ponderosa pine resin to D. brevicomis. In the first test the only significant difference was between 24 and 12 micrometer units, two dosages which should have been practically alike. In the third test the 1.9 milligram dosage caused significantly greater mortality with a 5-day exposure and yet there was no significant mortality at this dosage with a 7-day exposure.

The third tests weighings show that the factor of 0.15 is a fairly reasonable one. Thus the calculated vapor dosages in the first two tests were probably reasonably accurate. The weighings also show that once saturation has been reached, between 1.6 and 2.0 milligrams, there is no further vaporization of resin in the container.

In contrast to the few isolated cases of significant mortality, the great majority of the comparisons were nonsignificant. Visually the differences are slight.

Apparently the location of the beetle within the "stack" was of no significance.

Dendroctonus brevicomis with Jeffrey pine resin

Paralleling the three tests with D. brevicomis and ponderosa pine resin, three tests were made with this beetle and Jeffrey pine resin. The standard 4-oz. jar procedure was used. The resin was not weighed in the first test but weights were obtained in the other two.

In the first test micrometer units of 6, 12, 24, and 48 were used with a conversion factor of 0.08 milligrams of vapor for each micrometer unit. Thus the calculated vapor dosages, all well below saturation which is at about 20.0, were 0.5, 1.0, 1.9, and 3.8 milligrams. The effect of number of beetles per 4-oz. jar was studied by having 1 stack of 12 beetles in 3 jars and 2 stacks of 12 beetles each in 3 other jars at each dosage. The entire test was disassembled at 3 days and the replicates were held in a clean atmosphere for continued observations.

The results are summarized in table 9.

In the second test the micrometer units of resin used were 16, 32, and 64, along with a macro amount of 0.2 ml. Weighings were made of representative samples of these volumes at 48 hours to obtain the actual weight of resin vapor in the 4-oz. jar. There were 6 replicates of 12 beetles each for each dosage of the test. A complete series of replicates for each dosage was disassembled at 3, 5, and 7 days and the beetles retained in a clean atmosphere for observations at 2-day intervals. The results are summarized in table 10.

For the third test, volumes of resin were selected to give higher dosages of vapor than the two previous tests, by using micrometer units of 60, 120, 240, and 480. Weighings were made at 48 hours to obtain the actual weight of vapor in the 4-oz. jar. There were 4 replicates of 12 beetles each for each dosage with a complete series of replicates

Table 9.--Cumulative mortality of D. brevicomis, confined in 150 cc. volume, at different vapor dosages of Jeffrey pine resin

Stacks per 150 cc. volume	Micrometer units	Calculated weight of vapor	Total beetles used	Days after start of test 3	6	9	14
		Milligrams	Number	---Number---			
1	0	0.0	36	0	6	15	34
	6	0.5	36	3	9	18	34
	12	1.0	36	1	4	15	32
	24	1.9	36	1	5	17	34
	48	3.8	36	2	8	15	31
2	0	0.0	72	3	12	36	67
	6	0.5	72	3	12	30	68
	12	1.0	72	7	10	36	69
	24	1.9	72	5	10	28	67
	48	3.8	72	2	15	35	64

Table 10.--Cumulative mortality of D. brevicomis, confined in 150 cc. volume, at different vapor dosages of Jeffrey pine resin

Volume of resin[1]	Weight of resin	Weight of vapor	Total beetles used	Days after start of test 3	5	7	9	11
	--Milligrams--		Number	---Number---				
0	0.0	0.0	72	2	12	35	55	64
16 m.u.	7.1	2.7	72	3	16	37	53	68
32 m.u.	14.1	3.8	72	0	9	21	57	67
64 m.u.	29.1	5.3	72	7	18	33	57	67
0.2 ml.	226.4	22.4	72	72	-	-	-	-
0	0.0	0.0	72		13	32	53	70
16 m.u.	7.1	2.7	72		9	31	51	70
32 m.u.	14.1	3.8	72		11	28	43	64
64 m.u.	29.1	5.3	72		15	29	51	68
0.2 ml.	226.4	22.4	72		72	-	-	-
0	0.0	0.0	72			33	50	62
16 m.u.	7.1	2.7	72			18	42	66
32 m.u.	14.1	3.8	72			20	40	64
64 m.u.	29.1	5.3	72			29	48	59
0.2 ml.	226.4	22.4	72			72	-	-

1/ m.u. = micrometer units; ml. = milliliters.

Table 11.--Cumulative mortality of D. brevicomis, confined in a 150 cc. volume, at different vapor dosages of Jeffrey pine resin

Volume of resin	Weight of resin	Weight of vapor	Total beetles used	Days after start of test: 3	5	7	9	11
Micrometer units	--Milligrams--		Number	--Number--				
0	0.0	0.0	48	0	7	34	47	48
60	26.3	2.9	48	2	10	27	43	47
120	51.8	6.0	48	4	10	27	43	46
240	106.3	11.3	48	30	31	42	46	48
480	203.4	17.7	48	48	-	-	-	-
0	0.0	0.0	48		6	27	39	44
60	26.3	2.9	48		8	23	36	44
120	51.8	6.0	48		14	27	42	46
240	106.3	11.3	48		48	-	-	-
480	203.4	17.7	48		48	-	-	-

Table 12.--Location of dead (x) and living beetles (-) in fumigation chamber with certain dosages and exposure in table 11

Location in chamber	11.3 mg. at 3 days				6.0 mg. at 3 days				6.0 mg. at 5 days			
	Replicate #				Replicate #				Replicate #			
	1	2	3	4	1	2	3	4	1	2	3	4
Top	x	x	x	-	-	-	-	-	-	-	x	x
	x	x	-	-	-	-	-	-	-	x	x	-
	x	x	x	x	-	-	-	-	-	-	-	-
	x	x	-	-	-	-	-	-	-	-	-	-
Middle	x	-	x	-	-	x	-	x	x	-	-	x
	x	-	-	x	-	-	-	-	x	-	-	x
	x	x	x	-	-	-	-	-	x	x	x	-
	-	x	x	x	-	-	-	-	-	-	-	x
Bottom	-	x	-	-	-	-	-	-	-	-	-	-
	x	x	x	-	-	-	-	-	-	-	x	-
	-	x	x	x	-	x	-	x	-	-	-	x
	x	x	-	-	-	-	-	-	-	-	-	-

for each dosage disassembled at 3 and 5 days. Again the beetles were held in a clean atmosphere and observed every 2 days. The results are summarized in table 11. Table 12 is a detail presentation of the location of living and dead beetles in each replicate for the 11.3-milligram and 6.0-milligram dosage in which saturation should have been reached if there was stratification of the vapors.

Discussion.--At saturation, about 20.0 milligrams in 150 cc. volume, Jeffrey pine resin vapor is highly toxic to D. brevicomis. At subsaturation concentrations below 5.0 milligrams there is no toxicity, while at concentrations above 10.0 milligrams there is high toxicity. No data are available for concentrations between these two points.

In all tests, even where toxicity was encountered, there was little residual effect of the resin vapors; beetles which were alive when removed from a resinous atmosphere had a somewhat normal mortality rate during the subsequent period in a nonresinous atmosphere.

There is apparently no stratification of vapor in the 4-oz. jar.

A consideration of the weight of vapor produced by certain weights of resins indicated a much higher percent volatility than that given earlier in this report. The high percents were obtained with the lower weights. 7.1 milligrams yielded 2.7 milligrams or 38 percent; 14.1 milligrams yielded 3.8 milligrams or 27 percent; 29.1 milligrams yielded 5.3 milligrams or 18 percent. This property of Jeffrey pine resin makes it difficult to obtain the desired vapor weights.

Dendroctonus brevicomis with hybrid pine resin

In this general series of tests with D. brevicomis one test was made with the Jeffrey x ponderosa hybrid resin. Two volumes were used, 7 micrometer units to give a subsaturation dosage and 0.2 ml. to give a saturated one. Weighings were made of representative samples at 48 hours to get the vapor dosage. There were 4 replicates of 12 beetles each for each dosage with a complete series disassembled on the 3rd, 5th, and 7th days. The beetles were then retained in a clean atmosphere and were observed every 2 days. The results are summarized in table 13.

Discussion.--Hybrid resin vapors are toxic to D. brevicomis but only at saturation (about 5.4 milligrams in 150 cc.) and only when beetles are exposed for 7 days. As with Jeffrey pine resin, there appears to be little residual effect of an exposure of the beetle to the vapors which are toxic to a significant number of the beetles. It is of interest that the lowest vapor dosage of any of the three resins at which toxicity was obtained was with 5.4 mg. of hybrid pine resin. At this dosage Jeffrey pine resin was nontoxic; vapor dosages of this magnitude could not be obtained with ponderosa pine resin. The relationship of the three resins to the western pine beetle may be briefly summarized as follows:

	Sub-saturation	Saturation
ponderosa	nontoxic	nontoxic
hybrid	nontoxic	toxic
Jeffrey	toxic	toxic

Dendroctonus jeffreyi, with ponderosa, Jeffrey, and hybrid pine resin

Since previous work indicated that *D. jeffreyi* could not be handled with the standard fumigation test procedure, changes were necessary. The usual bundle of 4 fumigation cells was replaced with $\frac{1}{4}$-dram vials (figure 3g). The beetles were placed in individual vials which were then plugged with lumite screening. Otherwise the procedure was standard.

The first test was a comparison of the fumigation action of ponderosa and Jeffrey pine resin. Volumetric dosages of resin were selected to give both a saturated and nonsaturated atmosphere of each resin vapor. Exposures of 2, 3, and 4 days were used. However only the 2-day exposure will be reported since it was found that 10 beetles were too many for the 150 cc. volume of the 4-oz. jar for more than 2 days. There were 3 replicates of 10 beetles each for each dosage and exposure. The results are summarized in table 14.

The entire test was repeated just a few days later using only 5 beetles per 4-oz. jar and using exposures of 3, 5, and 7 days. There were 4 replicates for each dosage and exposure. The results are summarized in table 15.

A third test was made to determine the effect of hybrid resin. Again volumetric dosages were selected to give both a saturated and sub-saturated atmospheres. Exposures of 3, 5, and 7 days were used with 5 beetles per replicate and 3 replicates for each dosage and exposure. The results are summarized in table 16.

Table 13.--Cumulative mortality of D. brevicomis, confined in a 150 cc. volume, at different vapor dosages of hybrid pine resin

Volume of resin 1/	Weight of resin	Weight of vapor	Total beetles used	Days after start of test				
				3	5	7	9	11
	---Milligrams--		Number	---Number---				
0	0	0	48	3	8	24	37	46
7 m.u.	3.5	0.9	48	2	11	25	43	47
0.1 ml.	123.1	5.2	48	3	8	24	41	47
0	0	0	48		11	21	37	47
7 m.u.	3.5	1.5	48		7	21	35	45
0.1 ml.	123.1	5.4	48		16	28	40	46
0	0	0	48			12	29	39
7 m.u.	3.5	1.5	48			14	30	43
0.1 ml.	123.1	5.4	48			25	37	47

Comparison	t-value
0 vs. 4.3 mg.	.961 2.477

t at 90% = 1.895

1/ m.u. = micrometer units; ml. = milliliters.

Table 14.--Cumulative mortality of D. jeffreyi, confined in a 150 cc. volume, at different vapor dosages of ponderosa and Jeffrey pine resin

Resin	Volume 1/	Weight of resin	Weight of vapor	Total beetles used	Days after start of test				
					2	3	4	5	7
		--Milligrams--		Number	---Number---				
Ponderosa	6 m.u.	2.8	1.2	30	0	0	3	11	25
Ponderosa	0.2 ml.	230.5	2.0	30	4	5	14	21	28
Jeffrey	8 m.u.	3.7	1.2	30	0	1	3	14	21
Jeffrey	0.2 ml.	243.7	19.8	30	0	0	5	11	24
Untreated	0	0.0	0.0	30	0	0	6	15	27

1/ m.u. = micrometer units; ml. = milliliters.

Table 15.--Cumulative mortality of D. jeffreyi, confined in a 150 cc. volume, at different vapor dosages of ponderosa and Jeffrey pine resin

Resin	Volume[1]	Weight of resin	Weight of vapor	Total beetles used	Days after start of test: 3	5	7	9
		--Milligrams--		Number	---Number---			
Ponderosa	6 m.u.	3.2	1.1	20	0	11	17	20
				20		6	12	20
				20			19	20
	0.2 ml.	217.5	2.4	20	0	20	-	-
				20		17	20	-
				20			20	-
Jeffrey	8 m.u.	3.8	0.9	20	2	10	17	20
				20		11	17	20
				20			16	19
	0.2 ml.	189.2	19.9	20	2	3	9	13
				20		10	14	20
				20			20	-
Untreated	0	0	0	20	1	11	17	19
				20		10	19	20
				20			17	18

Comparisons		t-values
Untreated vs.	6 m.u. ponderosa	1.000
	.2 ml. "	2.330
	8 m.u. Jeffrey	.255
	.2 ml. "	.000
6 m.u. ponderosa vs.	.2 ml. ponderosa	3.661
	8 m.u. Jeffrey	1.276
	.2 ml. "	1.000
.2 ml. ponderosa vs.	8 m.u. Jeffrey	2.054
	.2 ml. "	2.330
8 m.u. Jeffrey vs.	.2 ml. Jeffrey	.255

t at 90% 1.833

1/ m.u. = micrometer units; ml.= milliliters.

Table 16.--Cumulative mortality of D. jeffreyi, confined in a 150 cc. volume, at different dosages of hybrid pine resin

Volume of resin[1/]	Weight of resin	Weight of vapor	Total beetles used	Days after start of test			
				3	5	7	9
	--Milligrams--		Number	---Number---			
7 m.u.	3.6	0.8	15	3	6	13	15
0.2 ml.	209.3	6.6	15	4	10	15	-
Untreated	0.0	0.0	15	2	8	13	19
7 m.u.	3.6	0.8	15		4	12	14
0.2 ml.	209.3	6.6	15		11	15	-
Untreated	0.0	0.0	15		2	10	14
7 m.u.	3.6	0.8	15			13	14
0.2 ml.	209.3	6.6	15			15	-
Untreated	0.0	0.0	15			13	15

1/m.u. = micrometer units; ml. = milliliters.

Discussion.--Because 10 beetles were found to be too many for the 4-oz. jar, the results of the first test must be reviewed with some caution. Nevertheless, the results of it and the second test strongly suggest that D. jeffreyi can tolerate a saturated atmosphere of Jeffrey pine resin vapors. Though the beetle was not markedly affected by subsaturation concentrations of ponderosa resin vapors, it apparently cannot tolerate a saturated atmosphere of this resin. Thus it appeared that each beetle, D. brevicomis and D. jeffreyi, could tolerate a saturated atmosphere of its host resin but could not tolerate a saturated atmosphere of a nonhost resin.

The third test indicated that D. jeffreyi cannot tolerate a saturated atmosphere of hybrid resin vapor over an extended period of time. At the same time it could tolerate subsaturation concentrations of this resin. Thus it is possible to see a parallel between the two beetles and the three resins. However, the data are not beyond some doubt. It would, therefore, seem advisable to design and conduct a test which would more firmly establish these relationships. Such facts could add very measurably to an understanding of the host relationship between pines and bark beetles and would also enable pine hybrids to be categorized with respect to bark beetles. At the present time their category is unknown.

These three tests with D. jeffreyi also illustrate some of the difficulties in work with fresh resin. In earlier tests the volatile fraction of ponderosa and Jeffrey pine resin was found to be 25 and 15 percent respectively. Yet in these last tests percentages of 40 and 30 were obtained where small amounts of resin were used. Again, it was not possible to determine the cause of these variations. Both inconsistencies in technique and variations in resin are suspected.

These three tests also indicate that the rate of natural mortality of D. jeffreyi is much faster than D. brevicomis. Such differences add to the difficulty of comparing the reactions of the two beetles.

Dendroctonus brevicomis with ponderosa resin and derivatives

The 1957 experiments indicated a distinct difference between the fumigant toxicity of the fresh resin of ponderosa pine and its liquid commercial derivative, turpentine. Since these results were obtained with the 30 cc. test tube technique, it was thought advisable to repeat the test with the 4-oz. procedure.

Prior to the actual test with beetles, weighings were made of 0.1 ml. samples of ponderosa resin, supernatant liquid, and turpentine to determine the vapor saturation of each in the 150 cc. volume of the 4-oz. jar. Out of curiosity Jeffrey pine resin and its supernatant liquid were also added to the test, though these two materials were not used in the subsequent fumigation tests. The results are summarized in table 17.

Following the loss-of-weight study, the test was made using D. brevicomis and the standard 4-oz. jar procedure. Volumetric dosages were selected to give both a saturated and subsaturated vapor concentration of the three ponderosa pine resin materials. Exposures of 3, 4, and 5 days were selected with 2 replicates of 12 beetles each for each dosage and exposure. The results are summarized in table 18.

Discussion.--The first test, involving the weighings, strongly indicated a distinct difference in the vapor saturation of ponderosa resin and turpentine. It likewise suggested an oxidizing action by turpentine. This oxidation caused an increase in the weight of the turpentine which in turn caused an apparent decrease in the weight of vapor in the confined 150 cc. volume. The results of the weighings along with the fumigation test with the beetles confirmed the previous contention that the supernatant liquid of resin and commercially prepared turpentine are not synonomous. The liquid and fresh resin were quite similar in both their vapor saturation and in the fumigant effect on D. brevicomis. There is no choice but to assume that the commercial preparation of turpentine changes the natural structure and properties of the constituents of fresh resin.

Effect of humidity

The 1957 experiments, using the 30 cc. test tube procedure, indicated a distinct relationship between humidity and the fumigation action of ponderosa and Jeffrey pine resin vapor. Experiments were made to ascertain this relationship using the 4-oz. jar procedure.

In the first test, using D. brevicomis, an atmosphere saturated with the vapor of each of the two resins was obtained with 0.1 ml. of resin in the 4-oz. jar. In the standard testing procedure the relative humidity was considered to be 70 percent. A humidity of 100 percent was obtained by placing a small vial of water with a cotton wick as

Table 17.--Loss of weight of resin and derivatives of ponderosa and Jeffrey pine in a 150 cc. confined atmosphere

Species	Resin or derivative	Volume	Days of confinement 1	2	3
		Milliliters	---Milligrams---		
Ponderosa	Fresh resin	0.1	1.8	1.8	1.6
	Supernatant 1 day	0.1	1.7	1.7	1.6
	" 1 week	0.1	1.4	1.7	1.5
	Turpentine	0.1	3.0	2.6	0.0
Jeffrey	Fresh resin	0.1	12.3	13.3	12.2
	Supernatant 1 week	0.1	12.9	13.0	12.3

an evaporative area in the 4-oz. jar. The standard procedure was used with 10 beetles, rather than the usual 12, per replicate. The exposures were 3 and 5 days with 2 replicates for each resin, humidity, and exposure. The results are summarized in table 19.

A second test was made using both toxic and nontoxic concentrations of Jeffrey pine resin vapor. There were two parts to the test based on length of exposure; in the first half the exposures were 1, 2, and 3 days; in the second half they were 3, 4, and 5 days. Thus the two parts encompassed exposures of 1 through 5 days. The results of this test are briefly summarized in table 20 in which is given only the mortality records at the time of disassembling the jars.

Discussion.--Contrary to the 1957 results, in which a 30 cc. test tube served as the fumigation chamber, there was no apparent relationship between humidity and the fumigant action of either ponderosa or Jeffrey pine resin. Similarly, humidity had no effect on natural mortality.

Comparison of 1957 and 1958 procedures

As the season progressed and as the results differed according to the procedure used, a series of three tests was made to compare the 1957 and 1958 techniques, using *D. brevicomis* and ponderosa pine resin at 70°F.±2°. In all tests the comparisons involved a 30 cc. cork-stoppered test tube and a 4-oz. jar with a teflon-sealed screw cap. The volumes in these two fumigation chambers were 30 cc. and 150 cc. respectively. The fumigation cells were the same for each chamber.

The first test attempted to compare the two devices using a saturated resin vapor atmosphere. The volumetric dosages were 1.0 ml. and 0.1 ml. for the 4-oz. jar and 0.1 ml. and 0.02 ml. for the test tube. Exposures of 3 and 5 days were used with 2 replicates of 8 beetles each. The results are summarized in table 21.

Table 18.--Cumulative mortality of D. brevicomis, confined in a 150 cc. volume, at different dosages of ponderosa pine resin and derivatives

Material	Volume	Weight of material	Weight of vapor	Total beetles used	Days after start of test: 3	4	5	6	7	8	9
	Micrometer units	--Milligrams--		--Number	---Number---						
Resin	6	3.5	0.7	24	2	2	6	10	17		
Supernatant	4	2.0	0.5	24	2	3	7	10	18		
Turpentine	2	1.5	0.8	24	2	6	8	15	21		
Untreated	0	0.0	0.0	24	1	1	3	5	18		
Resin	6	2.6	0.5	24		2	4	10	16		
Supernatant	4	2.6	0.8	24		5	8	11	16		
Turpentine	2	1.7	1.1	24		5	7	13	15		
Untreated	0	0.0	0.0	24		3	5	11	14		
Resin	6	3.9	0.6	24			2	9	12	18	
Supernatant	4	2.4	0.7	24			4	6	10	18	
Turpentine	2	2.2	1.4	24			2	4	13	21	
Untreated	0	0.0	0.0	24			7	10	13	18	
	Milliliters										
Resin	0.1	106.4	1.5	24	4	6	11	18	23		
Supernatant	0.1	72.7	1.6	24	5	8	12	18	21		
Turpentine	0.1	95.3	-0.7*	24	9	17	20	22	23		
Untreated	0.0	0.0	0.0	24	1	1	3	5	18		
Resin	0.1	105.6	1.8	24		11	11	15	20		
Supernatant	0.1	100.4	1.6	24		5	8	17	19		
Turpentine	0.1	96.0	-1.8*	24		15	18	21	23		
Untreated	0.0	0.0	0.0	24		3	5	11	14		
Resin	0.1	103.3	2.1	24			8	12	18	23	
Supernatant	0.1	73.7	1.7	24			13	16	21	23	
Turpentine	0.1	97.8	-2.3*	24			21	22	24	24	
Untreated	0.0	0.0	0.0	24			7	10	13	18	

* With .1 ml. of turpentine there was a gain in weight during the period of vaporization in the confined 150 cc. volume. Therefore, since all vapor weight are gotten by changing the sign of loss of weight of the initial material, these are given as minus values in this table.

Table 19.--Cumulative mortality of D. brevicomis confined with different resin vapor concentrations at different relative humidities in 150 cc. volume

Resin	Resin volume	Resin weight	Vapor weight	Approximate relative humidity	Total beetles used	Days after start of test 3	5	7	9	11
	Ml.	--Milligram--		Percent	Number	---Number---				
Ponderosa	.1	112.9	2.0	100	20	0	3	9	18	19
Ponderosa	.1	114.1	1.8	70	20	1	6	15	18	20
Jeffrey	.1	99.9	12.8	100	20	20	-	-	-	-
Jeffrey	.1	105.6	14.0	70	20	20	-	-	-	-
Untreated	0	0.0	0.0	100	20	1	8	13	17	18
Untreated	0	0.0	0.0	70	20	2	8	11	16	18
Ponderosa	.1	113.1	1.7	100	20		2	8	17	19
Ponderosa	.1	113.9	1.6	70	20		6	11	17	19
Jeffrey	.1	100.4	13.2	100	20		20	-	-	-
Jeffrey	.1	107.1	15.1	70	20		20	-	-	-
Untreated	0	0.0	0.0	100	20		2	9	12	18
Untreated	0	0.0	0.0	70	20		0	4	13	18

Table 20.--Mortality of D. brevicomis, confined in a 150 cc. volume, at 2 humidities with 2 dosages of Jeffrey pine resin vapor

Resin volume 1/	Resin weight	Vapor 2/ weight	Approximate relative humidity	Total beetles used	Days of confinement 1	2	3	4	5
	---Milligrams---		Percent	Number	---Number---				
0.1 ml.	90.1	12.4	100	30	0	6	10		
0.1 ml.	91.3	13.6	70	30	0	4	10		
32 m.u.	14.5	1.5	100	30	0	0	0		
32 m.u.	15.0	2.1	70	30	0	0	0		
Untreated	0.0	0.0	100	30	0	0	0		
Untreated	0.0	0.0	70	30	0	0	1		
0.1 ml.	131.2	15.2	100	30			10	10	10
0.1 ml.	129.8	15.4	70	30			10	10	10
32 m.u.	13.8	1.2	100	30			1	0	0
32 m.u.	13.8	2.0	70	30			1	1	0
Untreated	0.0	0.0	100	30			0	1	0
Untreated	0.0	0.0	70	30			0	0	1

1/ m.u. = micrometer units; ml. = milliliters.

2/ Average for the 3 days covered by each part of the test.

Table 21.--Cumulative mortality of D. brevicomis in saturated vapors of ponderosa pine resin in two types of fumigation chambers

Type of chamber	Volume of resin	Weight of resin	Weight of vapor	Total beetles used	Days after start of test: 3	5	7	9	11
	Milliliters	---Milligrams[1]		Number	---Number---				
4-oz. jar	1.0	967.2	1.9	16	3	9	14	16	
	0.1	110.6	1.8	16	3	9	14	16	
	0	0.0	0.0	16	1	6	12	14	
Test tube	0.1	- -	- -	16	6	13	16	16	
	0.02	20.2	1.8	16	3	7	15	16	
	0	0.0	0.0	16	3	4	9	13	
4-oz. jar	1.0	920.7	1.8	16		6	10	15	
	0.1	109.0	1.8	16		6	15	16	
	0	0.0	0.0	16		3	7	11	
Test tube	0.1	- -	- -	16		14	15	16	
	0.02	20.5	1.3	16		14	15	16	
	0	0.0	0.0	16		6	12	14	

1/ Weight of resin absorbed by cork was not determined for test tube values.

The second test was run shortly after the first one in an attempt to equalize the beetles and resin per volume of the chamber. Since the 4-oz. jar had 5 times the volume of the test tube, one-fifth as much resin and one-fifth as many beetles were placed in the test tube as in the 4-oz. jar. This required the use of 5 test tubes to make 1 replicate of 10 beetles. An effort was made to determine the actual amount of vapor in the test tube by substracting the cork weight-increase from the resin weight-loss. Exposures of 3 and 5 days were used with the beetles retained for subsequent observations. The results are summarized in table 22.

The third test was designed to test both a saturated and subsaturated vapor concentration by using the proper volumes of resin. Resin volumes of 1.6 ml., 0.4 ml., and 8 micrometer units were used in the 4-oz. jar while 0.1 ml. and 2 micrometer units were used in the test tube. A second series of 0.1 ml. volumes, having about $\frac{1}{4}$ the evaporative area as the standard resin vial was used in the test tube. The allocation of beetles and resin was the same as the previous test. Exposure of 3 and 5 days were used and the beetles were retained for subsequent observations. There were three replicates of 10 beetles each. The weighings were not completed because of a malfunctioning of the analytical balance. The results are summarized in table 23.

Table 22.--Cumulative mortality of D. brevicomis in saturated vapors of ponderosa pine resin in two types of fumigation chamber

Type of chamber	Volume of resin	Weight of resin	Weight of vapor	Total beetles used	Days after start of test 3	5	7	9
	Ml.	--Milligrams--		Number	---Number---			
4-oz.	0.5	475.1	3.5	30	10	20	28	30
	0.0	0.0	0.0	30	1	3	21	30
Test tube	0.1	113.9	0.7	30	15	26	30	30
	0.0	0.0	0.0	30	9	15	18	27
4-oz.	0.5	531.7	2.4	30		13	23	30
	0.0	0.0	0.0	30		6	15	27
Test tube	0.1	114.4	0.7	30		30	-	-
	0.0	0.0	0.0	30		9	21	27

Discussion.--It was clearly shown that the type of fumigation chamber can determine the fumigation effect of a resin. There were also indications that the type of chamber affects the rate of natural mortality even where the beetle-to-volume ratio was equal.

A comparison of the 4-oz. jar at saturation in the 3 different tests illustrates an unexplained variability between tests. In the first test, saturation was at 1.8 milligrams and there was no toxicity. In the second it was at 3.5 and 2.4 milligrams and there was toxicity while in the third it was at 2.8 and 2.4 milligrams without toxicity. In all cases there is an apparent toxicity of the resin at saturation in the test tube. This agrees with the 1957 and 1956 results in which the test tube procedure showed ponderosa resin vapors to be toxic to D. brevicomis.

Effect of fumigation on feeding

The last two fumigation tests of the season were a departure from the standard procedure. They were designed to determine the effect of semi-confined vapors of the three pine resins on the feeding activity of D. brevicomis.

The basic test device (figure 5) was a 4-inch length, 5 mm. i.d., of glass tubing into which a 2-inch length of ponderosa pine middle bark was forced. A glass elbow was attached to one end of the tube. The beetle was placed in the end of the tube near the bark and the opening was plugged with lumite screening. A vial of resin was attached to the other end of the elbow. When assembled the 4-inch tube was placed on an incline about 10° from the beetle toward the elbow. Mortality was observed and feeding activity was measured. A beetle was considered to have entered the bark if it was able to make a small niche. Each 2-inch piece of bark was considered 4 feeding units; a beetle chewing through $\frac{1}{4}$ of the piece was credited with 1 feeding unit.

Table 23.--Cumulative mortality of D. brevicomis in both saturated and subsaturated atmospheres of ponderosa pine resin vapors in two types of fumigation chambers

Type of container	Volume of resin 1/	Weight of resin	Weight of vapor 2/	Total beetles used	Days after start of test: 3	5	7	9	11
		---Milligrams---		Number	---Number---				
4-oz. jar	1.6 ml.	1,519.0	Sat.	30	1	12	19	29	
	0.4 ml.	487.1	2.8	30	2	14	28	30	
	8 mu.	3.8	1.4	30	2	8	18	29	
	0	0.0	0.0	30	2	10	19	29	
Test-tube	0.1 ml.	- -	Sat.	30	15	24	29	30	
	0.1 ml.	- -	Sat.	30	16	25	28	29	
	2 mu.	- -	Subsat.	30	3	11	22	29	
	0	- -	0.0	30	0	11	23	30	
4-oz. jar	1.6 ml.	- -	Sat.	30		10	17	29	
	0.4 ml.	- -	2.4	30		10	22	28	
	8 mu.	- -	1.4	30		6	18	27	
	0	- -	0.0	30		4	14	26	
Test-tube	0.1 ml.	- -	Sat.	30		26	30	--	
	0.1 ml.	- -	Sat.	30		25	30	--	
	2 mu.	- -	Subsat.	30		11	22	28	
	0	- -	0.0	30		12	20	27	

1/ m.u. = micrometer units; ml. = milliliters

2/ Sat. = Saturation, Subsat. = Subsaturation.

Comparison	t-values (90% = 2.015)
4 oz. at 0.4 ml. vs. test tube at 0.1 ml.	6.51
4 oz. at 0.4 ml. vs. test tube at 0.1 ml.	2.828
4 oz. at 8 mu. vs. test tube at 2 m.u.	.667
4 oz. check vs. test tube check	2.794

A dosage of 0.2 ml. of ponderosa, Jeffrey, and hybrid resin was used in the first test. The resin was replaced with a fresh supply each of the first three days of the test. There were 15 beetles per replicate and 2 replicates for each resin. Because some beetles escaped, there were less than 15 beetles in some of the replicates. The results are summarized in table 24.

In the second test the amount of resinous material was increased to 1.0 ml. and replenished each of the first 4 days of the test. The supernatant liquid of ponderosa pine resin was an added condition. Two types of checks were used; one was the standard which was considered 70 percent relative humidity;

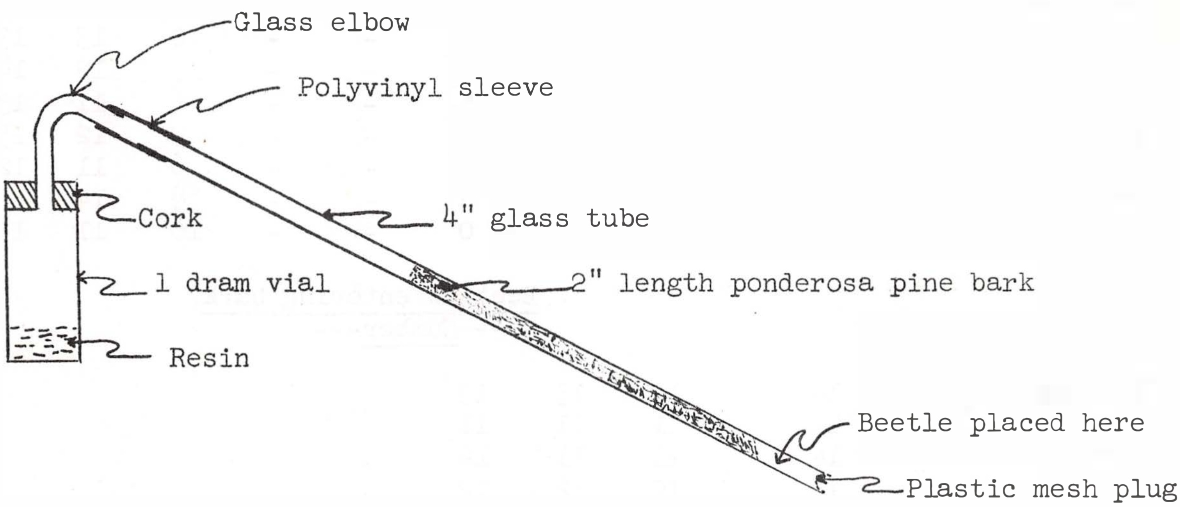


Figure 5.--Device for testing the feeding behavior of *D. brevicomis* in presence of resin vapors.

Table 24.--Cumulative mortality and feeding behavior of D. brevicomis in presence of semiconfined vapors from 0.2 ml. of three pine resins

Resin	Total beetles used	Days after start of test 1	2	3	4	5	6	7	8
		Mortality ---Number---							
Ponderosa	14	0	0	0	-	-	9	11	13
	14	0	0	0	-	-	10	13	13
Jeffrey	14	0	0	1	-	-	9	12	14
	15	0	0	0	-	-	6	11	13
Hybrid	15	0	0	2	-	-	9	12	15
	15	0	0	1	-	-	6	11	12
Untreated	15	0	0	3	-	-	8	14	15
	15	0	0	0	-	-	10	11	15
		Beetles entering bark ---Number---							
Ponderosa	14	12	13	13					
	14	11	11	11					
Jeffrey	14	11	11	12					
	15	10	12	12					
Hybrid	15	13	15	15					
	15	10	12	12					
Untreated	15	12	12	12					
	15	10	12	12					
		Amount of feeding ---Units1/---							
Ponderosa	14	14	21	28	-	-	34		
	14	12	17	22	-	-	28		
Jeffrey	14	11	17	22	-	-	29		
	15	10	12	19	-	-	24		
Hybrid	15	16	19	25	-	-	29		
	15	10	15	21	-	-	27		
Untreated	15	13	21	25	-	-	30		
	15	12	18	22	-	-	24		

1/ A maximum of 60 units for each replicate.

the other had a source of moisture in the resin vial which raised the humidity in the device to near 100 percent. There was only 1 replicate of 10 beetles each. The results are summarized in table 25.

Table 25.--Cumulative mortality and feeding behavior of *D. brevicomis* in the presence of semiconfined vapors of 1.0 ml. of three pine resins

Resin	Total beetles used	Days after start of test: 1	2	3	4	5	6	7
					Mortality ---Number---			
Ponderosa - resin	10	0	0	1	1	3	6	8
Ponderosa - liquid	10	0	0	0	1	6	10	10
Jeffrey - resin	10	0	0	0	1	5	8	10
Hybrid - resin	10	0	0	1	2	5	8	10
Untreated - 70% R.H.	10	0	0	0	1	5	9	10
Untreated - 100% R.H.	10	0	0	0	1	4	6	10
					Beetles entering bark ---Number---			
Ponderosa - resin	10		4	4	4			
Ponderosa - liquid	10		2	4	4			
Jeffrey - resin	10		3	3	4			
Hybrid - resin	10		1	1	1			
Untreated - 70% R.H.	10		5	6	6			
Untreated - 100% R.H.	10		6	6	6			

Discussion.--Despite the small size of the samples, it appeared that semiconfined resin vapors had little or no effect on either the rate of mortality or the feeding behavior of the beetle. One item of note was the apparent difference between the two lots of beetles used in the two tests. On the 6th day the mortality was 57 percent and 78 percent respectively for the first and second test. The number of beetles penetrating the bark by the 4th day was 85 percent and 42 percent respectively. This difference was not caused by the treatment since the untreated checks were almost as variable as the treatments. As in other tests, this indicated a considerable variation in the quality of the beetle from time to time.

CONTACT TOXICITY

The procedure used to conduct contact toxicity experiments was essentially the same as that used in 1957. The handling of beetles and resin prior to a test was the same as that used in the fumigation tests. To establish a test, fresh resin was placed into a short resin vial of 2.0 ml. capacity. Each beetle was removed from the gelatin capsule and immersed in the resin just long enough to be completely covered with resin. The beetle was quickly removed and placed on paper toweling and allowed to walk around for a minute so that the excess resin drained off. The treated beetle was placed in one end of a 4-inch, 5 mm. i.d., length of glass tubing in which a 2-inch length of ponderosa pine middle bark (between the phloem and corky bark) occupied the end nearest the beetle. This was the same as the 4-inch tube in figure 5. The ends of the tubing were plugged with small pieces of lumite screening. The assembled piece of tubing was placed at an angle of 10° upward from the beetle toward the bark. Observations were made daily for mortality and the feeding behavior of the beetles. Each 2-inch piece of bark was considered 4 feeding units; the amount of feeding was based on the proportion of this strip through which the beetle had bored.

The first experiment compared the effect of ponderosa and Jeffrey pine resin on *D. brevicomis*. The factors of humidity and footing for the beetle were added to the test. Footing was provided by a piece of lumite screening which followed the curvature of the glass tubing in the ¼-inch space between the end of the tube and the piece of bark. The thought was to give the beetle a better opportunity to start its boring action into the bark. Moisture was provided by attaching a vial of water with a wad of cotton as an evaporative surface to the 4-inch glass tube. This was attached to the upper end by a glass elbow. There were 20 beetles for each combination of resin, humidity, and footing. Thus there were 80 beetles for each resin. When the results were reviewed, it was found that neither humidity nor footing had any noticeable effect on the test. Therefore table 26 summarizes the test by each resin. Since some beetles escaped from the tube, somewhat less than 80 beetles are represented in the mortality data for each resin.

The second test was similar to the first except that *D. jeffreyi* was used and 7 mm. i.d. glass tube had to be used instead of the 5 mm. i.d. Initially there were 24 beetles for each of two resins and untreated check. However since a few escaped, table 27, which is a summary of the results, reports somewhat less than 24.

Discussion.--Both resins caused a significant increase in the mortality rate of both beetles. Jeffrey pine resin caused a greater increase in the mortality rate of *D. brevicomis* than did ponderosa pine resin. No such difference existed between the two resins on *D. jeffreyi*. Ponderosa resin either stimulated or enabled *D. brevicomis* to feed more extensively than untreated beetles. Jeffrey resin on the

Table 26.--Mortality and feeding behavior of D. brevicomis with a coating of fresh resin of ponderosa and Jeffrey pine

Resin	Total beetles used	Days after treatment 1	2	3	4	5	6	7
	Number	Cumulative mortality ---Percent---						
Ponderosa	70	6	10	26	63	79	93	99
Jeffrey	75	21	25	35	56	71	84	99
Untreated	69	0	4	17	33	62	79	99
Comparisons		t-values 1/						
Untreated vs. ponderosa		2.444	1.135	1.243	3.142			
" vs. Jeffrey		6.932	4.540	3.517	2.758			
Ponderosa vs. Jeffrey		3.142	5.976	2.538	.297			
		Attempted feeding ---Number---						
Ponderosa	80	66	67	67	67			
Jeffrey	80	12	29	36	36			
Untreated	80	72	73	73	75			
		Beetles entering bark ---Number---						
Ponderosa	80	53	57	59	59			
Jeffrey	80	5	13	24	26			
Untreated	80	24	33	37	39			
		Amount of feeding ---Units 2/---						
Ponderosa	80	85	126	146	160			
Jeffrey	80	6	24	41	46			
Untreated	80	44	82	98	104			

1/ t at 90% = 2.015.

2/ A possible maximum of 320 for each resin.

Table 27.--Mortality and feeding behavior of D. jeffreyi with a coating of fresh resin of ponderosa and Jeffrey pine

Resin	Total beetles used	Days after treatment 1	2	3	4	5	6
	Number	Cumulative mortality ---Percent---					
Ponderosa	19	5	11	32	63	89	100
Jeffrey	24	8	25	33	41	83	96
Untreated	23	0	0	0	0	43	70
		Attempted feeding ----Number----					
Ponderosa	24	23					
Jeffrey	24	21					
Untreated	24	24					
		Beetles entering bark -----Number-----					
Ponderosa	24	18					
Jeffrey	24	19					
Untreated	24	2					
		Amount of feeding ---Units 1/---					
Ponderosa	24	33	41	48	50		
Jeffrey	24	23	32	38	38		
Untreated	24	1	2	3	4		

1/ A possible maximum of 96 for each resin.

other hand had a general deleterious effect in this respect. Both ponderosa and Jeffrey pine resin either stimulated or enabled D. jeffreyi to feed more extensively than untreated beetles. The results suggest some effect by resins which is other than deleterious to the beetle.

STOMACH TOXICITY

A very small test was made to see how D. brevicomis reacted to bark impregnated with various resinous materials. The standard 2-inch lengths of ponderosa pine middle bark were the basic test pieces. These were dipped in the fresh resin and supernatant liquid of

ponderosa, Jeffrey and the hybrid pine, as well as in commercially prepared turpentine of ponderosa pine resin. Half of the sections of bark were impregnated with the 7 materials 8 to 10 hours before the test was assembled. These sections were allowed to dry in the interim. The other half of the sections were treated immediately before use, though the excess material on the bark was blotted off. The treated 2-inch sections of bark were placed in 4-inch lengths of 5 mm. i.d. glass tubing so that one end of the bark was about $\frac{1}{4}$-inch inside one end of glass tube. This was the same as the 4-inch tube in figure 5. A beetle was placed in the end nearest the bark and both ends of the glass tube were plugged with lumite mesh. The assembled tubes were placed on a 10° slope from the beetle toward the strip of bark. The results of the mortality observations are given in table 28, while table 29 is a record of the feeding behavior.

Discussion.--Two treatments, turpentine and untreated, differ quite radically from all the rest. When turpentine was applied to the bark within $\frac{1}{2}$ hour of use it caused an excessive rate of mortality. The attempted feedings and amount of feeding with these two treatments were far greater than the rest. Thus it would appear that natural resinous materials inhibit feeding but cause no increase in mortality. However turpentine, a processed resinous material, both stimulated feeding and caused an increase in mortality. The results again point to a distinct difference between turpentine and natural resinous materials.

Table 28.--Cumulative mortality of D. brevicomis in tubes containing treated bark

Material	Time applied to bark before use	Total beetles used	Days after treatment 1	2	3	4	5	6
	Hours	Number	---Number---					
Ponderosa - resin	8-10	10	0	0	2	6	-	8
Jeffrey - resin		10	0	0	2	6	-	10
Hybrid - resin		10	0	0	1	5	-	10
Ponderosa - supernatant		10	0	1	2	3	-	8
Jeffrey - supernatant		10	0	0	4	7	-	9
Hybrid - supernatant		10	0	1	2	6	-	10
Ponderosa - turpentine		10	1	1	3	5	-	9
Untreated		10	1	1	2	4	-	9
Ponderosa - resin	$\frac{1}{2}$	10	0	1	4	7	-	10
Jeffrey - resin		10	0	0	0	1	-	5
Hybrid - resin		10	0	1	2	5	-	6
Ponderosa - supernatant		10	0	1	4	7	-	9
Jeffrey - supernatant		10	0	0	1	3	-	9
Hybrid - supernatant		10	0	0	1	3	-	9
Ponderosa - turpentine		10	0	8	9	9	-	10
Untreated		10	0	0	3	6	-	9

Table 29.--Feeding behavior of D. brevicomis in tubes containing treated bark

Material	Time applied to bark before use	Total beetles used	Days after treatment 1	2	3	1	2	3	4
	Hours	Number	Attempted feeding --Number--			Amount of feeding --Units[1]--			
Ponderosa - resin	8-10	10	3	3	3	1	2	2	2
Jeffrey - resin		10	2	4	7	1	2	4	4
Hybrid - resin		10	2	2	5	2	2	2	2
Ponderosa - supernatant		10	1	1	3	0	1	2	2
Jeffrey - supernatant		10	2	5	6	0	3	7	7
Hybrid - supernatant		10	0	0	1	0	0	1	1
Ponderosa - turpentine		10	5	6	6	5	9	9	9
Untreated		10	8	8	9	8	14	17	18
Ponderosa - resin	½	10	2	2	5	1	3	5	5
Jeffrey - resin		10	1	1	2	0	1	2	4
Hybrid - resin		10	1	1	3	0	2	3	3
Ponderosa - supernatant		10	4	6	7	4	4	6	6
Jeffrey - supernatant		10	1	2	6	0	0	5	8
Hybrid - supernatant		10	1	1	3	1	2	3	4
Ponderosa - turpentine		10	7	7	7	5	6	6	6
Untreated		10	9	9	10	5	12	16	17

1/ A possible maximum of 40 units per treatment.